

WHAT IS CLAIMED IS:

1. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide having a flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase catalytic activity.

2. The isolated polynucleotide of claim 1, wherein said nucleotide sequence is selected from the group consisting of DNA and RNA.

3. The isolated polynucleotide of claim 1, wherein said nucleotide sequence is selected from the group consisting of complementary DNA, genomic DNA and messenger RNA.

4. The isolated polynucleotide of claim 1, further comprising a vector, wherein said nucleotide sequence is ligated to said vector.

5. The isolated polynucleotide of claim 4, wherein said vector is an expression vector.

6. The isolated polynucleotide of claim 5, wherein said nucleotide sequence is ligated to said vector in an orientation selected from the group consisting of a sense orientation and an antisense orientation.

7. The isolated polynucleotide of claim 5, wherein said vector is selected from the group consisting of a vector propagatable in plant cells and a vector propagatable in a microorganism cell.

8. The isolated polynucleotide of claim 5, wherein said nucleotide sequence is as set forth in SEQ ID NO:20 or a functional part thereof.

9. The isolated polynucleotide of claim 1, wherein said nucleotide sequence shares between 50 and 100 % identical bases with SEQ ID NO:20 or a functional part thereof, as determined using a sequence analysis software package developed by the Genetic Computer Group

(GCG) at the University of Wisconsin with gap creation penalty of 50 and gap extension penalty of 3.

10. The isolated polynucleotide of claim 1, wherein said nucleotide sequence is hybridizable with SEQ ID NO:20 or a functional part thereof under hybridization selected from the group consisting of stringent hybridization, moderate hybridization and mild hybridization, wherein stringent hybridization is effected by a hybridization solution of 6 x SSC and 1 % SDS, hybridization temperature of 65 °C, final wash solution of 0.1 x SSC and final wash at 60 °C, moderate hybridization is effected by a hybridization solution of 6 x SSC and 1 % SDS, hybridization temperature of 58 °C, final wash solution of 0.5 x SSC and final wash at 50 °C, whereas mild hybridization is effected by a hybridization solution of 6 x SSC and 1 % SDS, hybridization temperature of 50 °C, final wash solution of 2 x SSC and final wash at 40 °C.

11. The isolated polynucleotide of claim 1, wherein said polypeptide is as set forth in SEQ ID NO:21 or a functional part thereof.

12. The isolated polynucleotide of claim 1, wherein said polypeptide shares between 50 and 100 % identical or conserved amino acids with SEQ ID NO:21 or a functional part thereof, as determined using a sequence analysis software package developed by the Genetic Computer Group (GCG) at the University of Wisconsin with gap creation penalty of 12 and gap extension penalty of 4.

13. The isolated polynucleotide of claim 1, wherein said nucleotide sequence originates from a species of the genus *Citrus*.

14. A plant cell of a plant species naturally expressing a flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase messenger RNA, wherein the plant cell is genetically modified to include, in an expressible antisense orientation, a nucleotide sequence encoding an antisense RNA molecule being capable of *in vivo* base pairing with said flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase messenger RNA, to thereby render said flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase messenger RNA, when expressed, amenable to degradation by nucleases present in the plant cell.

15. The plant cell of claim 14, wherein said nucleotide sequence is selected from the group consisting of a complementary DNA and a genomic DNA.

16. The plant cell of claim 14, wherein said nucleotide sequence is extrachromosomal.

17. The plant cell of claim 14, wherein said nucleotide sequence is intrachromosomal.

18. The plant cell of claim 14, wherein said nucleotide sequence is ligated to an expression vector in antisense orientation.

19. The plant cell of claim 18, wherein said nucleotide sequence is as set forth in SEQ ID NO:20 or a portion thereof longer than 15 nucleotides.

20. The plant cell of claim 14, wherein said nucleotide sequence shares between 80 and 100 % identical bases with SEQ ID NO:20 or a portion thereof longer than 15 nucleotides, as determined using a sequence analysis software package developed by the Genetic Computer Group (GCG) at the University of Wisconsin with gap creation penalty of 50 and gap extension penalty of 3.

21. The plant cell of claim 14, wherein said nucleotide sequence is hybridizable *in vitro* with SEQ ID NO:20 or a portion thereof longer than 15 nucleotides under hybridization selected from the group consisting of stringent hybridization, moderate hybridization and mild hybridization, wherein stringent hybridization is effected by a hybridization solution of 6 x SSC and 1 % SDS, hybridization temperature of 65 °C, final wash solution of 0.1 x SSC and final wash at 60 °C, moderate hybridization is effected by a hybridization solution of 6 x SSC and 1 % SDS, hybridization temperature of 58 °C, final wash solution of 0.5 x SSC and final wash at 50 °C, whereas mild hybridization is effected by a hybridization solution of 6 x SSC and 1 % SDS, hybridization temperature of 50 °C, final wash solution of 2 x SSC and final wash at 40 °C.

22. The plant cell of claim 14, wherein said nucleotide sequence originates from a species of the genus *Citrus*.

23. The plant cell of claim 14, wherein said species is selected from the group consisting of grapefruit and pomelo.

24. A transgenic plant of a species naturally expressing a flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase messenger RNA, wherein the transgenic plant is genetically modified to include, in an expressible antisense orientation, a nucleotide sequence encoding an antisense RNA molecule being capable of *in vivo* base pairing with said flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase messenger RNA, to thereby render said flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase messenger RNA, when expressed, amenable to degradation by nucleases present in the transgenic plant.

25. The transgenic plant of claim 24, wherein said nucleotide sequence is selected from the group consisting of a complementary DNA and a genomic DNA.

26. The transgenic plant of claim 24, wherein said nucleotide sequence is extrachromosomal.

27. The transgenic plant of claim 24, wherein said nucleotide sequence is intrachromosomal.

28. The transgenic plant of claim 24, wherein said nucleotide sequence is ligated to an expression vector in antisense orientation.

29. The transgenic plant of claim 28, wherein said nucleotide sequence is as set forth in SEQ ID NO:20 or a portion thereof longer than 15 nucleotides.

30. The transgenic plant of claim 24, wherein said nucleotide sequence shares between 80 and 100 % identical bases with SEQ ID NO:20 or a portion thereof longer than 15 nucleotides, as determined using the sequence analysis software package developed by the Genetic Computer

Group (GCG) at the University of Wisconsin with gap creation penalty of 50 and gap extension penalty of 3.

31. The transgenic plant of claim 24, wherein said nucleotide sequence is hybridizable *in vitro* with SEQ ID NO:20 or a portion thereof longer than 15 nucleotides under hybridization selected from the group consisting of stringent hybridization, moderate hybridization and mild hybridization, wherein stringent hybridization is effected by a hybridization solution of 6 x SSC and 1 % SDS, hybridization temperature of 65 °C, final wash solution of 0.1 x SSC and final wash at 60 °C, moderate hybridization is effected by a hybridization solution of 6 x SSC and 1 % SDS, hybridization temperature of 58 °C, final wash solution of 0.5 x SSC and final wash at 50 °C, whereas mild hybridization is effected by a hybridization solution of 6 x SSC and 1 % SDS, hybridization temperature of 50 °C, final wash solution of 2 x SSC and final wash at 40 °C.

32. The transgenic plant of claim 24, wherein said nucleotide sequence originates from a species of the genus *Citrus*.

33. The transgenic plant of claim 24, wherein said species is selected from the group consisting of grapefruit and pomelo.

34. A cell genetically modified to include, in an expressible sense orientation, a nucleotide sequence encoding a flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase.

35. The cell of claim 34, wherein said nucleotide sequence is selected from the group consisting of complementary DNA and genomic DNA.

36. The cell of claim 34, wherein said nucleotide sequence is extrachromosomal.

37. The cell of claim 34, wherein said nucleotide sequence is intrachromosomal.

38. The cell of claim 34, wherein said nucleotide sequence is ligated to an expression vector in sense orientation.

39. The cell of claim 38, wherein said nucleotide sequence is as set forth in SEQ ID NO:20 or an active part thereof.

40. The cell of claim 34, wherein said nucleotide sequence shares between 50 and 100 % identical bases with SEQ ID NO:20 or an active part thereof, as determined using the sequence analysis software package developed by the Genetic Computer Group (GCG) at the University of Wisconsin with gap creation penalty of 50 and gap extension penalty of 3.

41. The cell of claim 34, wherein said nucleotide sequence is hybridizable *in vitro* with SEQ ID NO:20 or an active part thereof under hybridization selected from the group consisting of stringent hybridization, moderate hybridization and mild hybridization, wherein stringent hybridization is effected by a hybridization solution of 6 x SSC and 1 % SDS, hybridization temperature of 65 °C, final wash solution of 0.1 x SSC and final wash at 60 °C, moderate hybridization is effected by a hybridization solution of 6 x SSC and 1 % SDS, hybridization temperature of 58 °C, final wash solution of 0.5 x SSC and final wash at 50 °C, whereas mild hybridization is effected by a hybridization solution of 6 x SSC and 1 % SDS, hybridization temperature of 50 °C, final wash solution of 2 x SSC and final wash at 40 °C.

42. The cell of claim 34, wherein said nucleotide sequence originates from a species of the genus *Citrus*.

43. The cell of claim 34, wherein the cell is of a microorganism.

44. The cell of claim 43, wherein said microorganism is producing activated rhamnose.

45. The cell of claim 43, wherein said microorganism is selected from the group consisting of *Lactobacillus* and *Saccharomyces*.

46. The cell of claim 34, wherein the cell is of a plant species.

47. The cell of claim 46, wherein said plant species is of a genus selected from the group consisting of *Citrus*, *Nicotiana*, *Vitis* and *Daucus*.

48. A commercial process of producing neohesperidin comprising the steps of:

- (a) obtaining hesperidin;
- (b) treating said hesperidin with a hesperidinase, thereby obtaining hesperetin-7-glucoside; and
- (c) treating said hesperetin-7-glucoside with a flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase in the presence of activated rhamnose, thereby obtaining neohesperidin.

49. The commercial process of claim 48, wherein said activated rhamnose is selected from the group consisting of UDP-rhamnose or dTDP-rhamnose.

50. The commercial process of claim 48, wherein said hesperidinase is immobilized on a solid support, whereas said hesperidin is treated with said hesperidinase while passing over said solid support.

51. The commercial process of claim 48, wherein said treatment of hesperetin-7-glucoside with a flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase in presence of activated rhamnose of step (c) is effected *in vivo* by a cell genetically modified to overexpress said flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase, which said cell is producing activated rhamnose and is capable of intake of said hesperetin-7-glucoside.

52. The commercial process of claim 51, further comprising the step of extracting said neohesperidin from said cell.

53. A commercial process of producing neohesperidin dihydrochalcone comprising the steps of:

- (a) obtaining hesperidin;
- (b) treating said hesperidin with a hesperidinase, thereby obtaining hesperetin-7-glucoside;
- (c) treating said hesperetin-7-glucoside with a flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase in the

presence of activated rhamnose, thereby obtaining neohesperidin;

- (d) treating said neohesperidin with an alkali, thereby obtaining neohesperidin chalcone; and
- (e) reducing said neohesperidin chalcone, thereby obtaining neohesperidin dihydrochalcone

54. The commercial process of claim 53, wherein said activated rhamnose is selected from the group consisting of UDP-rhamnose or dTDP-rhamnose.

55. The commercial process of claim 53, wherein said hesperidinase is immobilized on a solid support, whereas said hesperidin is treated with said hesperidinase while passing over said solid support.

56. The commercial process of claim 53, wherein said treatment of hesperetin-7-glucoside with a flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase in presence of activated rhamnose of step (c) is effected *in vivo* by a cell genetically modified to overexpress said flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase, which said cell is producing activated rhamnose and is capable of intake of said hesperetin-7-glucoside.

57. The commercial process of claim 56, further comprising the step of extracting said neohesperidin from said cell prior to treating said neohesperidin with said alkali.

58. A method of modifying a rhamnose-1-6-glucose linkage of a chemical compound to a rhamnose-1-2-glucose linkage comprising the steps of:

- (a) enzymatically treating said chemical compound to remove a rhamnose group thereof, thereby obtaining a first derivative chemical compound having a terminal glucose group; and
- (b) enzymatically treating said first derivative with recombinant flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase in a presence of activated rhamnose, thereby obtaining a second

derivative chemical compound having said
rhamnose-1-2-glucose linkage.

59. The method of claim 58, wherein said activated rhamnose is selected from the group consisting of UDP-rhamnose or dTDP-rhamnose.

60. The method of claim 58, wherein enzymatically treating said first derivative with a flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase in said presence of activated rhamnose is effected *in vivo* by a cell genetically modified to overexpress said flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase, which said cell is producing activated rhamnose and is capable of intake of said first derivative.

61. The method of claim 60, further comprising the step of extracting said second derivative from said cell.

62. A plant cell of a plant species naturally expressing a flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase, wherein the plant cell is genetically modified to include, in an overexpressible sense orientation, a nucleotide sequence encoding a sense RNA molecule being capable of inducing a co-suppression effect to thereby reduce production of said flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase in the plant cell.

63. The plant cell of claim 62, wherein said nucleotide sequence is selected from the group consisting of a complementary DNA and a genomic DNA.

64. The plant cell of claim 62, wherein said nucleotide sequence is extrachromosomal.

65. The plant cell of claim 62, wherein said nucleotide sequence is intrachromosomal.

66. The plant cell of claim 62, wherein said nucleotide sequence is ligated to an expression vector in sense orientation.

67. The plant cell of claim 66, wherein said nucleotide sequence is as set forth in SEQ ID NO:20 or an active part thereof.

68. The plant cell of claim 62, wherein said nucleotide sequence shares between 50 and 100 % identical bases with SEQ ID NO:20 or an active part thereof, as determined using a sequence analysis software package developed by the Genetic Computer Group (GCG) at the University of Wisconsin with gap creation penalty of 50 and gap extension penalty of 3.

69. The plant cell of claim 62, wherein said nucleotide sequence is hybridizable *in vitro* with SEQ ID NO:20 or an active part thereof under hybridization selected from the group consisting of stringent hybridization, moderate hybridization and mild hybridization, wherein stringent hybridization is effected by a hybridization solution of 6 x SSC and 1 % SDS, hybridization temperature of 65 °C, final wash solution of 0.1 x SSC and final wash at 60 °C, moderate hybridization is effected by a hybridization solution of 6 x SSC and 1 % SDS, hybridization temperature of 58 °C, final wash solution of 0.5 x SSC and final wash at 50 °C, whereas mild hybridization is effected by a hybridization solution of 6 x SSC and 1 % SDS, hybridization temperature of 50 °C, final wash solution of 2 x SSC and final wash at 40 °C.

70. The plant cell of claim 62, wherein said nucleotide sequence originates from a species of the genus *Citrus*.

71. The plant cell of claim 62, wherein said species is selected from the group consisting of grapefruit and pomelo.

72. A transgenic plant of a species naturally expressing a flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase, wherein the plant is genetically modified to include, in an overexpressible sense orientation, a nucleotide sequence encoding a sense RNA molecule being capable of inducing a co-suppression effect to thereby reduce production of said flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase in the plant cell.

73. The transgenic plant of claim 72, wherein said nucleotide sequence is selected from the group consisting of a complementary DNA and a genomic DNA.

74. The transgenic plant of claim 72, wherein said nucleotide sequence is extrachromosomal.

75. The transgenic plant of claim 72, wherein said nucleotide sequence is intrachromosomal.

76. The transgenic plant of claim 72, wherein said nucleotide sequence is ligated to an expression vector in sense orientation.

77. The transgenic plant of claim 76, wherein said nucleotide sequence is as set forth in SEQ ID NO:20 or an active part thereof.

78. The transgenic plant of claim 72, wherein said nucleotide sequence shares between 50 and 100 % identical bases with SEQ ID NO:20 or an active part thereof, as determined using a sequence analysis software package developed by the Genetic Computer Group (GCG) at the University of Wisconsin with gap creation penalty of 50 and gap extension penalty of 3.

79. The transgenic plant of claim 72, wherein said nucleotide sequence is hybridizable *in vitro* with SEQ ID NO:20 or an active part thereof under hybridization selected from the group consisting of stringent hybridization, moderate hybridization and mild hybridization, wherein stringent hybridization is effected by a hybridization solution of 6 x SSC and 1 % SDS, hybridization temperature of 65 °C, final wash solution of 0.1 x SSC and final wash at 60 °C, moderate hybridization is effected by a hybridization solution of 6 x SSC and 1 % SDS, hybridization temperature of 58 °C, final wash solution of 0.5 x SSC and final wash at 50 °C, whereas mild hybridization is effected by a hybridization solution of 6 x SSC and 1 % SDS, hybridization temperature of 50 °C, final wash solution of 2 x SSC and final wash at 40 °C.

80. The transgenic plant of claim 72, wherein said nucleotide sequence originates from a species of the genus *Citrus*.

81. The transgenic plant of claim 72, wherein said species is selected from the group consisting of grapefruit and pomelo.

82. A plant cell of a plant species naturally expressing a flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase, the plant cell being genetically modified to knock-out a gene encoding said flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase to thereby abolish production of said flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase in the plant cell.

83. A transgenic plant of a plant species naturally expressing a flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase, the transgenic plant being genetically modified to knock-out a gene encoding said flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase to thereby abolish production of said flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase in the transgenic plant.